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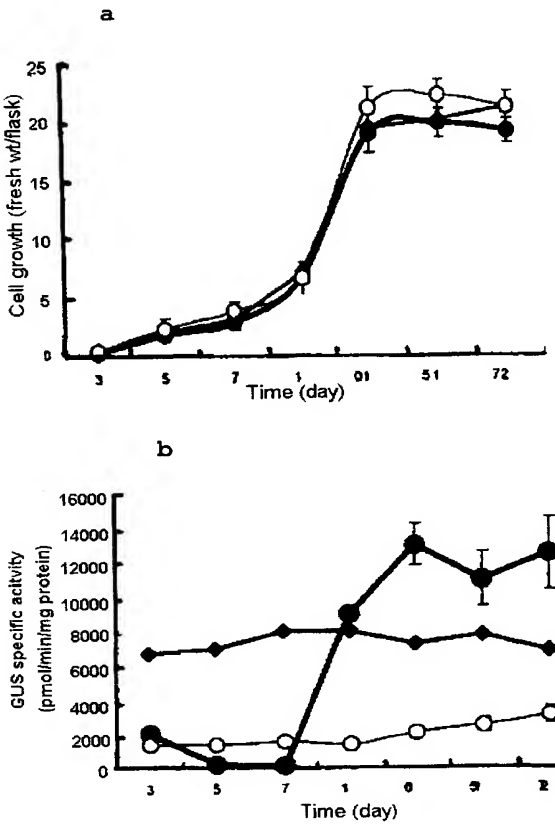
(54) Title: A PEROXIDASE GENOMIC GENE DERIVED FROM *IPOMOEA BATATAS* AND A PROMOTER THEREOF

(57) Abstract: The present invention relates to stress inducible promoter. Particularly, it relates to the new genomic gene coding peroxidase isoenzyme derived from *Ipomoea batatas* and the promoter thereof of which the expressions are strongly induced under environmental stresses in the cultured cells and whole plants. The whole or the part of the peroxidase promoter of the present invention can be effectively used to develop stress-tolerant plants which have resistance to environmental stresses, and to develop transformed organisms which can produce useful materials on a large scale.



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A peroxidase genomic gene derived from *Ipomoea batatas* and a promoter thereof

FIELD OF THE INVENTION

The present invention relates to a stress inducible promoter. Particularly, it relates to a new genomic gene coding a peroxidase isoenzyme of *Ipomoea batatas* and a promoter thereof whose expressions are strongly induced under environmental stresses in the cultured cells and whole plants.

The whole or part of the peroxidase gene promoter of the present invention can be effectively used to develop stress-tolerant plants, resistant to the environmental stresses, and to develop transformed organisms producing useful materials on a large scale.

BACKGROUND

When the most organisms including plants are exposed to various environmental stresses generated according to the environmental aggravation of earth as well as biological stress of bacteria, insect and virus, oxygen which is necessary for keeping life changes into reactive oxygen species of superoxide anion radical, hydrogen peroxide and hydroxyl radical that induce

serious physiological disorder. Therefore, there are many systems in the body to get rid of these reactive oxygen species such as the macromolecular antioxidative enzymes of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) and small molecular antioxidative materials of vitamin C, vitamin E and glutathione.

It has been well known that peroxidase widely exists in plant cells as a reducing enzyme which reduces hydrogen peroxide in the presence of electron donor. Peroxidase is becoming the center of interest since it has an important role for plant to react on the various external stresses and is an industrially important enzyme by being used as various clinical test reagents because of its sensitive enzyme reaction. Generally, the activity of plant peroxidase is increased by the various environmental stresses and, particularly, is very high in the cultured cells which are considered to be grown under the high oxidative stress. It has been reported that the cultured cell of *Ipomoea batatas* produces peroxidase on a large scale than any other cultured cells of plant (*Phytochemistry*, 39, 981-984, 1995).

The genes coding peroxidase isoenzymes of the some plants that are originated from about 20 species plants of horseradish, barley, wheat, rape, tobacco, spinach and rice have been reported. The present inventors have isolated the peroxidase gene of *Ipomoea*

batatas for the first time. We have reported that the anionic peroxidase *swpa1* and neutral peroxidase *swpn1* isolated from the cultured cells of *Ipomoea batatas* are specifically expressed in the cultured cells and stem of *Ipomoea batatas,* and plurally exist in genome (*Mol. Gen. Genet.*, 255, 382-391, 1997). It has also reported that peroxidase can be produced on a large scale by transforming the whole or part of these peroxidase genes to plant organisms and cells (*Phytochemistry*, 47, 695-700, 1998; *Phytochemistry* 48, 1287-1290, 1998).

In addition, the present inventors have found out the nucleic acid sequence of the anionic peroxidase gene *swpa2* (GeneBank Accession NO. AF109124) and *swpa3* (GeneBank Accession No. AF109123) from *Ipomoea batatas*. According to this, *swpa2* has 71 signal peptides, *swpa3* has 66 signal peptides, and *swpa2* and *swpa3* have 1245 and 1310 bp of nucleic acid sequences coding 358 and 349 of amino acids, respectively. The isoelectric point of mature protein expressed by *swpa2* and *swpa3* is 4.1 and 4.3, respectively, and this shows that all the genes code the anionic peroxidase. AAUAA of typical polyadenylation signal and poly(A)-tail exist in the 3'-untranslated region of *swpa2* and *swpa3,* and particularly, the N-terminal sequence of *swpa2* gene is completely same as that of major isoenzyme (A-2) from the cultured cells of *Ipomoea batatas*. In addition, the

present inventors have demonstrated that *swpa2* gene is strongly expressed in response to wounding, low temperature or ozone treatment in the leaf of *Ipomoea batatas*, on the other hand, *swpa3* gene is weakly expressed in response to wounding, but strongly expressed by low temperature or ozone treatment (*Mol. Gen. Genet.*, 261, 941-947, 1999).

SUMMARY OF THE INVENTION

It is an object of this invention to provide a genomic peroxidase DNA originated from *Ipomoea batatas* and nucleic acid sequence thereof.

It is a further object of this invention to provide a promoter of which expression is strongly induced by the various environmental stresses.

It is an additional object of this invention to provide transformed organisms that are resistant to the various environmental stresses and a preparing method thereof.

It is also an object of this invention to provide transformed organisms that are capable to produce useful materials on a large scale and a preparing method thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a result analyzed by Southern blot for isolating genomic DNA including peroxidase gene of *Ipomoea batatas* of the present invention.

FIG. 2a shows nucleic acid sequences of the genomic gene SWPA2 coding peroxidase of *Ipomoea batatas* of the present invention and amino acid sequences coded therefrom.

FIG. 2b is continued from the nucleic acid sequences of genomic DNA SWPA2 coding peroxidase and the amino acid sequences coded therefrom of the FIG. 2a.

FIG. 3 shows nucleic acid sequences of the promoter of genomic DNA SWPA2 coding peroxidase of *Ipomoea batatas*.

FIG. 4 shows a schematic view of preparing promoter deletion mutants of genomic DNA SWPA2 coding peroxidase of *Ipomoea batatas*.

FIG. 5 shows a result of transit assay using the promoter deletion mutants of the present invention.

FIG. 6 shows a result measuring GUS activity of the transformed yeast which is introduced with the promoter deletion mutant of the present invention.

FIG. 7a shows a result measuring the induced GUS activity in the absence of wounding to the transformed tobacco plants which are introduced with the promoter deletion mutants of the present invention.

FIG. 7b shows a result measuring the induced GUS activity in the presence of wounding to the transformed tobacco plants which are introduced with the promoter deletion mutants of the present invention.

FIG. 8a shows a result measuring the GUS activity in the absence of H_2O_2 treatment to the transformed tobacco plants which are introduced with the promoter deletion mutants of the present invention.

FIG. 8b shows a result measuring the induced GUS activity in the presence of H_2O_2 treatment of transformed tobacco plants which are introduced with the promoter deletion mutants of the present invention.

FIG. 9a shows a result measuring the GUS activity in the absence of UV irradiation to the transformed tobacco plants which are introduced with the promoter deletion mutants of the present invention.

FIG. 9b shows a result measuring the induced GUS activity in the presence of UV irradiation of transformed tobacco plants which are introduced with the promoter deletion mutants of the present invention

FIG. 10a shows the callus induced from the transformed tobacco plants which are introduced with the promoter deletion mutants of the present invention after GUS staining.

A; pBS1314 B; pBS1824

C; control D; pBI121

FIG. 10b shows results measuring the GUS activity

of the callus induced from the transformed tobacco plants which are introduced with the promoter deletion mutants of the present invention.

A; pBS1314 B; pBS1824

C; control D; pBI121

FIG. 11a shows a cell growth curve of the suspension cultured cells induced from the transformed tobacco plants which are introduced with the promoter deletion mutants of the present invention.

FIG. 11b shows a result of measuring the GUS activity of the suspension cultured cells induced from the transformed tobacco plants which are introduced with the promoter deletion mutants of the present invention.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

A terminology and technology referred in the present detailed description are used as general meaning of the technical field which includes the present invention. In addition, references mentioned in the present detailed description are all included in the present detailed description for describing the present invention.

"Derivatives of nucleic acid sequences" of the present invention mean modified nucleic acid sequences

by substitution, deletion or addition of one or more base in the nucleic acid sequence of *swpa2*, SWPA2 or SWPA2 promoter with keeping its biological activity.

"Derivatives of proteins" of the present invention mean modified amino acid sequences by substitution, deletion or addition of one or more amino acid in the amino acid sequence coded by *swpa2* with having peroxidase activity.

"SWPA2 promoter" means a nucleic acid sequence which includes the nucleic acid sequence represented by the SEQ. ID NO.2 and gives a transcription activity to genes operably linked thereto under the appropriate condition.

"Active fragment of SWPA2 promoter" means a nucleic acid sequence which includes the part of nucleic acid sequence represented by the SEQ. ID NO.2 and gives SWPA2 promoter activity to genes operably linked thereto.

"Transformed organisms" mean transformed cells or plants that are transformed with the DNA construct comprising SWPA2 promoter operably linked to a DNA sequence coding for a heterologous protein. The transformed organisms of the present invention include transformed microorganisms, animal cells, plant cells, transgenic animals or plants and cultured cells derived from them.

"Environmental stress" means biotic or abiotic

stresses such as wounding, reactivate oxygen species, tumor, heat, moisture, temperature, salt, air pollution, UV, heavy metal, et al. which functions to objective organism as a stress.

Hereinafter, the present invention is described in detail.

The present invention provides the genomic gene SWPA2 coding peroxidase originated from *Ipomoea batatas* and nucleic acid sequence thereof.

The SWPA2 of the present invention comprises the whole or part of nucleic acid sequences represented by the SEQ. ID NO.1, wherein the DNA sequence includes exons coding peroxidase *swpa2* of *Ipomoea batatas*.

The SWPA2 is a genomic clone having the same ORF (open reading frame) as *swpa2* by third screening from the genomic DNA library of *Ipomoea batatas* and named as natural SWPA2 (see FIG. 2).

The natural SWPA2 comprises 3 exons, 2 introns and promoter region, and the nucleic acid sequences of its exons are completely same as those of *swpa2* cDNA (Gene Bank Accession No. AF109124). The peroxidase genomic clone of *Ipomoea batatas* comprises considerably long intron that, especially, the first intron of them is 737 bp. It is longer than 100 - 300 bp of intron in any other plant. The each intron of the peroxidase genomic clone follows GT-AG rule which 5' end of intron

starts with GT and 3′ of intron ends with AG.

In addition, the present invention provides the promoter of which expression is induced by various environmental stresses.

In this specification, SWPA2 promoter is used as the meaning including SWPA2 promoter and an active fragment thereof unless any special limitation is described. SWPA2 promoter of the present invention comprises the whole or part of nucleic acid sequences represented by the SEQ. ID NO.2 having promoter activity. For an example, SWPA2 promoter preferably comprises 503 to 1828 of nucleic acid sequence represented by the SEQ. ID NO.2 or the part DNA sequence thereof having promoter activity.

The promoter according to the present invention is strongly expressed by environmental stresses and derived from the natural SWPA2 of genomic peroxidase gene of *Ipomoea batatas*.

The natural SWPA2 has promoter region in the upstream of translation initiation site and is named as SWPA2 promoter. The characteristics on the nucleic acid sequence of SWPA2 promoter are analyzed by Transcription Element Search Software (TESS) of Computational Biology & Informatics Laboratory. As a result, SWPA2 promoter comprises nucleic acid sequences

represented by the SEQ. ID NO.2, and has CAAT box on -895 position and TATA box for transcription initiation (see FIG. 3).

As a result of sequence analysis, it has been found that SWPA2 promoter contained regulatory elements of eucaryotic promoter, i.e. TATA box for transcription initiation and CAAT box at -895 position. In addition, SWPA2 promoter has a similar motif to G box represented by NNNSACGTGNCM at -445 to -455 region which is a binding site of transcriptional regulatory protein and regulated by ABA (abscisic acid), methyl jasmonate, UV, wounding and hypoxia (Williams, M. et al., 1992) (see Fig. 3). Transcription factor SP-1 which is expressed tissue specifically and can be induced by stress, exists between G box of SWPA2 promoter and transcription initiation site. Furthermore, 6 repeat sequence of AAAATAA was found in the SWPA2 promoter region.

SWPA2 promoter also has heat shock element (HSE) containing consensus sequence of AGAAN at -1170 to -1188 region (see Fig. 3). GCN-4 and AP-1 has been known to respond reactive oxygen species, and especially AP-1 is known as essential element to respond nitrogen at C-hordein promoter of barley (Muller, M. et al., 1993). In addition, there are oct-1 and C/EBP beta for enhancer element in SWPA2 promoter. GCN-4 is in the

three places and AP-1 in the two places. Especially, there are inverted repeat sequences of GCN-4 and AP-1 between -1175 and -1163 region (see Fig. 3)

The expression of SWPA2 promoter of the present invention is strongly induced by various external factors including oxidative stress. Particularly, since it is strongly induced in cultured cell, SWPA2 promoter of the present invention can be useful for the development of environmental stress-tolerant plants and the production of useful materials using transformed plant cells.

The SWPA2 promoter of the present invention can effectively induce the expression of gene by stresses. For this, the promoter of the present invention comprises various transcription factors which recognize the stresses by ABA, methyl jasmonate, wounding, hypoxia, heat or nitrogen. By using these characteristics, it can be used for manufacturing fusion gene construct which comprises DNA sequence having promoter activity and structural gene operably linked to this DNA sequence. Since the fusion gene construct comprises the structural genes related to the production of useful materials and SWPA2 promoter gene, and expresses the useful material by regulation of SWPA2 promoter under various environmental stresses, it can be useful for manufacturing transformed organisms for the production of useful material. In addition, if

the structural genes are related to various environmental stress resistance in the fusion gene construct, it can be used for manufacturing stress-tolerant organisms which are resistant to external stress.

The promoter of the present invention is functional in microorganism as well as plant, and therefore, it can be used for developing transformed plant cells, transformed plants and transformed callus derived therefrom, transformed microorganisms and transformed animal cells.

In addition, the present invention provides a preparing method of transformed organisms using the SWPA2 promoter which can induce the production of useful material by various environmental stress.

The preparing method of transformed organisms comprises the steps of;

1) constructing an expression vector which comprises the first DNA sequence representing promoter activity which comprises nucleic acid sequence represented by the SEQ. ID NO.2 or the part thereof operably linked to the second DNA sequence coding the heterologous protein,
2) introducing the expression vector into a host cell; and
3) selecting the host cell introduced with the

expression vector.

In the preparing method of the present invention, the useful material contains various proteins or peptides representing pharmacological effect and a material endowing stress resistance to transformants. Therefore, the preparing method of the present invention is used for developing stress-tolerant organisms and producing the useful materials in transformed organisms.

EXAMPLES

Practical and presently preferred embodiments of the present invention are illustrative as shown in the following Examples.

However, it will be appreciated that those skilled in the art, on consideration of this disclosure, may make modifications and improvements within the spirit and scope of the present invention.

Example 1: Analysis of peroxidase genomic DNA

To find genomic DNA of peroxidase gene *swpa2*, the present inventors performed Southern blot analysis of *swpa2* gene to confirm that it actually existed in the *Ipomoea batatas* genome. 15 μg of genomic DNA was

extracted by the method of Dellaporta et al. (Dellaporta, *Newsletter*, 57, 26-29, 1983), from the cultured cells of *Ipomoea batatas*, digested with restriction enzymes *Eco*RI, *Hinc*II and *Hind*III, and performed agarose gel electrophoresis. After transferring genomic DNA on the gel to the nylon membrane, hybridization was carried out using the gene fragment labeled with ^{32}P at the specific 3'-end untranslated region of *swpa2* gene (Fig. 1).

As illustrated in Fig. 1, *swpa2* gene was detected more than 2 bands. It implies that *swpa2* gene exists plurally on the separate genome.

Example 2: Isolation and sequencing analysis of peroxidase genomic DNA

To isolate genomic DNA containing peroxidase gene *swpa2* of the present invention, the present inventors performed the experiment as following.

The genomic DNA library of *Ipomoea batatas* was prepared using λ Blue STAR™ *BamH*I Arms vector kit (Novagen). After that, PCR was performed with *swpa2*-specific primer pairs using the genomic DNA library as a template. 0.5 kb of PCR product was amplified,

labeled with ^{32}P, and used for genomic DNA library screening of peroxidase in *Ipomoea batatas*. The genomic DNA library screening was carried out by the method of Sambrook et al (Molecular cloning: a laboratory manual 2ed. 1989). After third library screening, a genomic DNA clone having the same open reading frame (ORF) as *swpa2* was obtained and named as natural SWPA2.

Natural SWPA2 had approximately 4 kb of nucleic acid sequence represented by the SEQ. ID NO.1 and consisted of 3 exons, two introns and its promoter region (Fig. 2). It was confirmed that the nucleic acid sequence of its exon was completely the same as that of *swpa2* cDNA sequence. The first intron of peroxidase genomic clone in *Ipomoea batatas* was 737 bp in size considerably longer than other plant species of 100 to 300 bp in size, and both introns followed the GT-AG rule beginning 5'-end with GT and ending 3'-end with AG.

Example 3: Promoter analysis of peroxidase genomic DNA SWPA2

The promoter of natural SWPA2 consisted of nucleic acid sequence represented by the SEQ. ID NO.2 from translation initiation site to -1824 bp region of the SWPA2 gene (Fig. 3). Sequence characteristics of the

SWPA2 promoter was analyzed using Transcription Element Search Software (TESS) of Computational Biology & Informatics Laboratory.

As a result of sequence analysis, it was found that SWPA2 promoter contained regulatory elements of eucaryotic promoter, i.e. TATA box for transcription initiation and CAAT box at -895 position. It was also found a similar motif to G box represented by NNNSACGTGNCM at -445 to -455 region which was a binding site of transcriptional regulatory protein and was regulated by ABA, methyl jasmonate, UV, wounding and hypoxia (Williams, M. et al., 1992)(Fig. 3). Transcription factor SP-1, which was expressed tissue-specifically and could be induced by stress, existed between G box of SWPA2 promoter and transcription initiation site. In addition, 6 repeat sequence of AAAATAA was found.

SWPA2 promoter also had heat shock element (HSE) containing AGAAN consensus sequence at -1170 to -1188 region (Fig. 3). GCN-4 and AP-1 were known to respond reactive oxygen species, and especially AP-1 was known to essential element to respond nitrogen at C-hordein promoter of barley (Muller, M. et al., 1993). In addition, there were oct-1 and C/EBP beta for enhancer element in SWPA2 promoter. GCN-4 was in the three places and AP-1 in the two places. Especially, there

were inverted repeat sequences of GCN-4 and AP-1 between -1175 and -1163 region (Fig. 3).

In result, SWPA2 promoter of the present invention contained various stress-recognizing elements including reactive oxygen species and could be used for developing stress-tolerant plants, and for developing the industrial cell lines to produce useful materials under stress culture conditions.

Example 4: Preparation of deletion mutant of SWPA2 promoter

To make deletion mutant of SWPA2 promoter, the present inventors performed PCR to amplify SWPA2 promoter region using Ex Taq polymerase (Takara) and sequence-specific primers. The sequence-specific primers consisted of upstream primers represented by the SEQ. ID NO.3 to 7 and downstream primer represented by the SEQ. ID NO.8. All the upstream primers were constructed to contain *SalI* restriction site and the downstream primer to contain *BamHI* restriction site. The deleted mutants amplified by PCR using the primer pairs were 1824, 1314, 968, 602 and 354 bp, respectively (Fig. 4).

After digestion of the resulting PCR products with

SalI/BamHI restriction enzyme, DNA fragments were subcloned into pBI101 plasmid vector (Clontech) which contained GUS coding region and NOS transcription terminator as binary vector. After that, plasmid vector pBS1824, pBS1314, pBS968, pBS602 and pBS354 were prepared to contain -1824, -1314, -968, -602 and -354 deletion construction, respectively, and they were used for transit assay.

Example 5: Transit assay of SWPA2 promoter using tobacco protoplasts

Transit assay using deletion mutants of SWPA2 promoter was performed as following.

First, suspension cultured cells of tobacco BY-2 (*Nicotiana tabacum* L. cv. Bright yellow 2) were subcultured for 3 days. After that, cells were treated with enzyme solution containing 2% cellulase R-10 and 0.5% macerozyme for 3 hours to separate their protoplasts. After transfection of deletion mutant plasmid vectors prepared by the Example 4 into the protoplasts using polyethylene glycol method, the protoplasts were cultured in the darkness at 25℃ for 16 hours. Fluorescence of protoplasts containing deletion mutant plasmid vector was measured using

method of Jefferson et al. (*Plant Mol. Biol. Ref.*, 5, 387-405, 1987), and promoter activities were calculated by the produced amount of GUS protein.

As a result of transit assay, it was found that especially when SWPA2 promoter containing -1314 deletion construction was used, GUS activity was increased more than 30 times compared with the case of using CaMV 35S promoter (Fig. 5).

Example 6: Expression of SWPA2 promoter in yeast

To investigate whether SWPA2 promoter is expressed in yeast *Saccharomyces cerevisiae*, the present inventors used yeast/*E. coli* shuttle vector Yep352 (Hill et al., 1986) and *S. cerevisiae* L3262 as a host. Each plasmid vector containing deletion mutant of SWPA2 promoter prepared by the Example 4 which was fused with GUS gene and NOS terminator, was introduced into Yep352 vector, and was transformed into *S. cerevisiae* by yeast transformation method using PEG and lithium acetate. After culturing the transformed yeast in SD/URA$^-$ medium (minimal SD base-UraDO (drop out) supplement, Clontech), promoter activity was investigated by measuring fluorescence generated from the transformed yeast via the same method of the Example 5.

As a result, in case of transformed yeast introduced with -1314, -1620 and -1824 deletion construction, GUS activity was increased 1.6, 1.4 and 8.4-fold compared to that of using CaMV 35S promoter, respectively (Fig. 6).

Example 7: GUS expression using SWPA2 promoter in the transgenic plants and its cultured cells

<7-1> Test plants and the preparation of transgenic plants

Tobacco plant (*Nicotiana tabacum* cv. Xanthi) was used for plant transformation. It was transformed with *Agrobacterium fumefaciens* LBA4404 which was introduced with plasmid vector pBS1824 (-1824 deletion construction), pBS1314 (-1314 deletion construction) containing deletion mutant of SWPA2 promoter and pBI121 containing GUS gene fused to CaMV 35S promoter, respectively. Transgenic plants were selected by culturing the transformed tobacco in MS medium (Murashige T. et al., *Physiol Plant*, 15, 473~497, 1962) containing 200 mg/l of kanamycin and 300 mg/l of claforan. After rooting and shooting step, the transgenic plants were moved into little flowerpot for growing and used for experiments.

To investigate whether the deletion mutant of SWPA2 promoter was introduced correctly into the transgenic plants, PCR was performed using NPTII primer pairs represented by SEQ. ID NO.9 and 10 and promoter primer pairs represented by SEQ. ID NO.11 and 12. In case of using NPTII primer pairs, PCR was performed 30 cycles of 95℃ for 1 min, 65℃ for 1 min and 72℃ for 1 min, and in case of using promoter primer pairs, it was performed 30 cycles of 95℃ for 1 min, 62℃ for 1 min and 72℃ for 1 min.

As a result, DNA fragment of 0.7 kb in size by NPTII primer pairs and 1.0 kb in size by promoter primer pairs were detected in the transgenic plants. Therefore, it was demonstrated that foreign genes were correctly incorporated into the transgenic plants.

<7-2> Preparation of transformed cell

To prepare transformed cells, the leaf of transgenic plants of which gene incorporation was confirmed by the Example <7-1>, was cultured in MS medium containing 0.1 mg/l BAP, 2 mg/l NAA and 30 g/l sucrose to induce callus formation. As a result of this, suspension culture of transformed tobacco cell line induced from the callus was established.

Transgenic tobacco cells of the present invention which was transformed with plasmid vector pBS1314 (-1314 deletion construction), was deposited at Korean Collection for Type Culture of Korea Research Institute of Bioscience and Biotechnology on October, 16, 2000 (Accession No: KCTC 0875BP).

<7-3> Measurement of GUS expression in the transgenic plants induced by stress

To investigate the expression pattern of SWPA2 promoter in the transgenic plants induced in response to external environmental stress, GUS activity induced in response to stress was measured after treatment of wounding, H_2O_2, or UV to the transgenic plants.

First, to investigate the expression pattern of SWPA2 promoter by wounding, GUS activity was measured after hurting the transgenic plants. In result, there was no change of GUS expression in the transgenic plants introduced with pBI121 vector containing CaMV 35S promoter-GUS gene. However, in case of the transgenic plants introduced with pBS1824 and pBS1314, there was increase of GUS expression after 3 days of wounding treatment (Fig. 7). In case of pBS1314-transgenic plants, GUS expression induced in response

to wounding was increased about 3.6-fold compared with untreated control plants. Although the GUS expression of pBS1824-transgenic plants was lower than that of pBS1314 transgenic plants, the expression pattern of pBS1824-transgenic plants induced in response to wounding was similar to that of pBS1314-transgenic plants.

In addition, to investigate the expression pattern of SWPA2 promoter by H_2O_2 treatment, 7 mm diameter of leaf disks prepared from well-grown leaf was floated on 1 mM H_2O_2 solution and cultured under continuous light. After cultivation, the expression pattern of SWPA2 promoter induced in response to H_2O_2 treatment was investigated by measuring GUS activity.

In result, after 48 hours of cultivation, the GUS expression of pBS1314-transgenic plants was increased 5.8-fold compared to untreated control and 1.7-fold compared with CaMV 35S-transgenic plants (Fig. 7). In case of transgenic plants incorporated with pBS1824 vector, GUS expression was increased 3.2-fold by H_2O_2 treatment and 1.2-fold compared with CaMV 35S promoter transgenic case.

Furthermore, after UV irradiation onto the transgenic tobacco plants incorporated with deletion mutant of genomic peroxidase gene SWPA2 promoter, GUS

activity induced in response to UV was measured. As a result, after 24 hours of cultivation, GUS activity of pBS1314-transgenic plants was increased about 5.6-fold compared with untreated control and about 1.2-fold compared with CaMV 35S promoter-transgenic plants (Fig. 8). There was 2.5-fold increase of GUS expression by UV irradiation in the transgenic plants introduced with pBS1824 vector.

<7-4> The GUS expression of callus and suspension culture

To investigate whether the expression of SWPA2 promoter of the present invention could be regulated with regard to cell growth, GUS activity of transformed callus derived from the transgenic plants which was introduced with pBS1314, pBS1824 and PBI121 vectors, respectively, was measured.

In result, pBS1314-transformed callus showed 4-fold higher GUS activity than pBI121-transformed callus (Fig. 10a and 10b)

In addition, as a result of investigating changes of GUS activity in the suspension cultured cell derived from the transformed callus, transformed callus introduced with pBS1314, pBS1824 and pBI121 showed the

same growth pattern each other and reached plateau after 15 days of cultivation (Fig. 11a and 11b). The pBI121-transformed cells maintained relatively low level of GUS expression irrelevant to cell growth. The pBS1824-transformed cells also maintained constant level of GUS expression irrelevant to cell growth, but showed higher GUS expression than pBI121-transformed cells. On the other hand, in case of pBS1314-transformed cells, the GUS expression was maintained lowly for 5 and 7 days of cultivation, but was increased rapidly after 7 days of cultivation. After 15 days of cultivation, maximum level of GUS expression was observed and maintained until the end of cultivation time.

INDUSTRIAL APPLICABILITY

The present invention provides a new peroxidase genomic gene SWPA2 and a promoter thereof from *Ipomoea batatas* which are expressed strongly under environmental stress conditions. The whole or part of the promoter of the present invention is used to develop stress-tolerant plants resistant to environmental stresses and transformed organisms of cells, plants, microorganisms, etc., producing useful materials on a large scale.

Those skilled in the art will appreciate that the conceptions and specific embodiments disclosed in the foregoing description may be readily utilized as a basis for modifying or designing other embodiments for carrying out the same purposes of the present invention. Those skilled in the art will also appreciate that such equivalent embodiments do not depart from the spirit and scope of the invention as set forth in the appended claims.

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSE OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT

issued pursuant to Rule 7.1

TO : KWAK, Sang-Soo
Expo Apt. 307-306, #464-1, Jeonmin-dong, Yusong-ku, Taejon 305-390, Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
BS1314 (*Nicotiana tabacum* cv. Xanthi)	KCTC 0875BP

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:
[x] a scientific description
[ ] a proposed taxonomic designation
(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on **October 16 2000.**

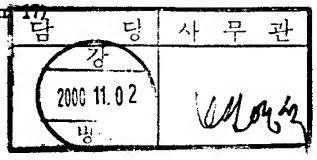
IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: **Korean Collection for Type Cultures** Address: Korea Research Institute of Bioscience and Biotechnology (KRIBB) #52, Oun-dong, Yusong-ku, Taejon 305-333, Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority of authorized official(s): BAE, Kyung Sook, Director Date: **October 18 2000**

Form BP/4 (KCTC Form 11)



What is Claimed is

1. Genomic DNA sequence comprising the whole or part of nucleic acid sequence represented by the SEQ. ID NO.1 which codes peroxidase encoded by *swpa2* nucleic acid sequence or its derivatives.

2. DNA sequence representing promoter activity which comprises nucleic acid sequence represented by the SEQ. ID NO.2 or the part thereof.

3. DNA sequence according to claim 2, wherein the promoter activity is induced by environmental stresses.

4. DNA sequence according to claim 3, wherein the environmental stress is induced by wounding, reactive oxygen species, heat, moisture, temperature, salt, air pollution, UV-light or heavy metal.

5. DNA construct comprising the first DNA sequence representing promoter activity which comprises nucleic acid represented by the SEQ. ID NO.2 or the part thereof operably linked to the second DNA sequence coding a heterologous protein.

6. DNA construct according to claim 5, wherein the first DNA sequence further contains at least one factor recognizing stresses caused by ABA, methyl jasmonate, wound, hypoxia, reactive oxygen species, heat or nitrogen.

7. A preparing method of transformed organisms producing a heterologous protein which comprises the steps of:

1) Constructing an expression vector which comprises the first DNA sequence representing promoter activity which comprises nucleic acid sequence represented by the SEQ. ID NO.2 or the part thereof operably linked to the second DNA sequence coding the heterologous protein,

2) introducing the expression vector into a host cell; and

3) selecting the host cell transfered with the expression vector.

8. The preparing method according to claim 7, wherein the heterologous protein is selected from the group consisting of various proteins or peptides representing pharmacological effect and materials endowing stress-resistance to transformed organisms.

9. The preparing method according to claim 7, wherein

the host cell is plant cell, animal cell or microorganism.

10. The preparing method according to claim 7, wherein the transformed organism is microorganism, plant cell, plant or callus derived therefrom

11. A transformed tobacco callus prepared by the preparing method of claim 7 (Accession NO.; KCTC 0875BP).

FIGURES

FIG. 1

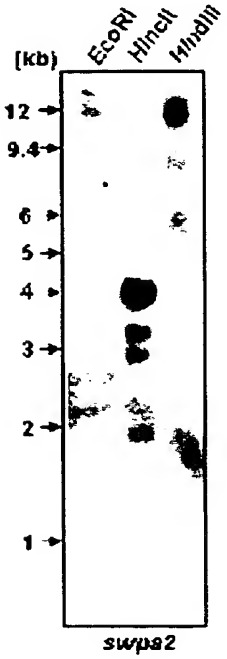
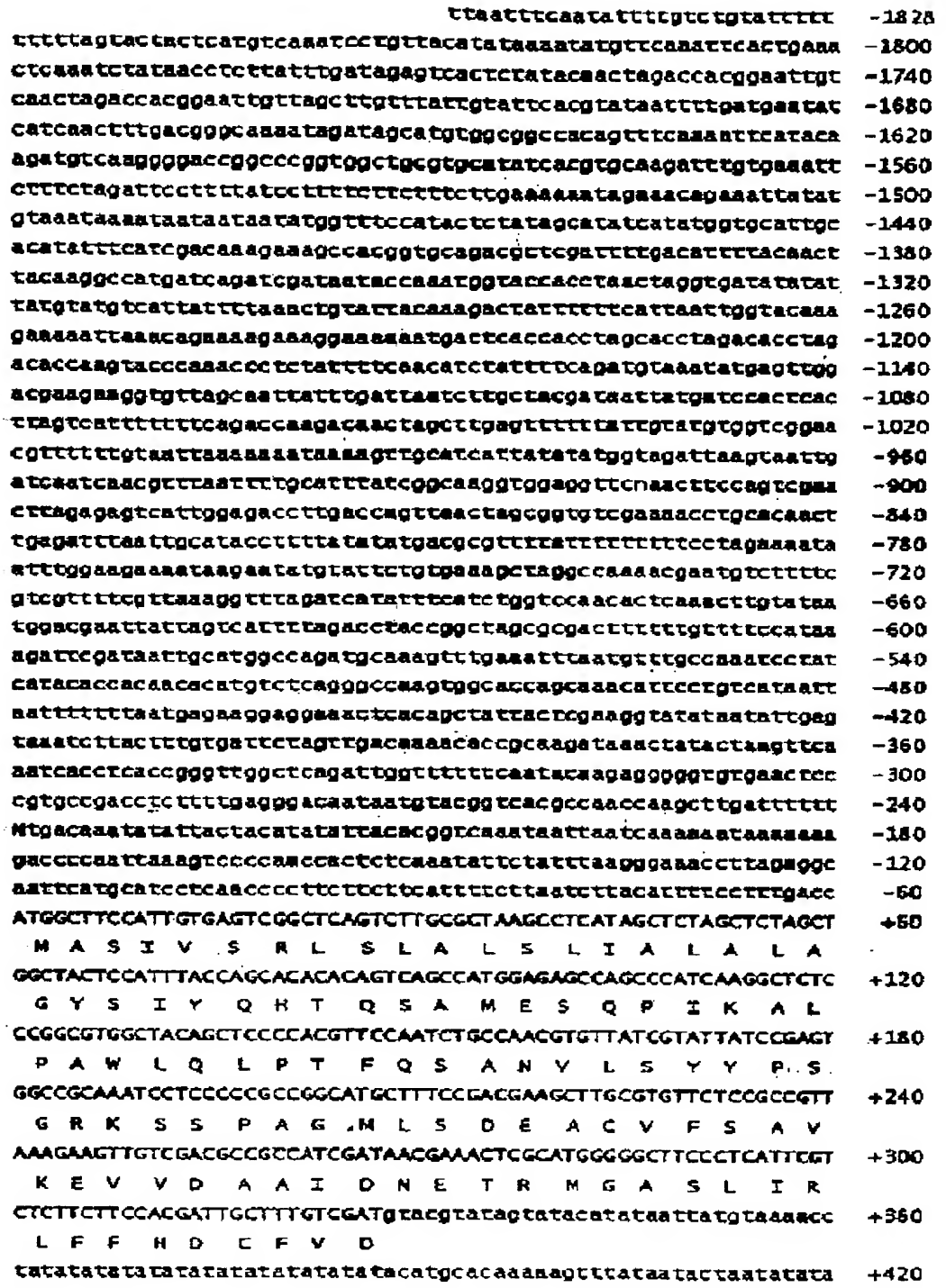


FIG. 2a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M A S I V S R L S L A L S L I A L A L A
GGCTACTCCATTTACCAGCAGACACAGTCAGCCATGGAGAGCCAGCCCATCAAGGCTCTC +120
G Y S I Y Q H T Q S A M E S Q P I K A L
CCGGCGTGGCTACAGCTCCCCACGTTCCAATCTGCCAACGTGTTATCGTATTATCCGAGT +180
P A W L Q L P T F Q S A N V L S Y Y P S
GGCCGCAAATCCTCCCCCGCCGGCATGCTTTCCGACGAAGCTTGCGTGTTCTCCGCCGTT +240
G R K S S P A G M L S D E A C V F S A V
AAAGAAGTTGTCGACGCCGCCATCGATAACGAAACTCGCATGGGGGCTTCCCTCATTCGT +300
K E V V D A A I D N E T R M G A S L I R
CTCTTCTTCCACGATTGCTTTGTCGATgtacgtatagtatacatataattatgtaaaacc +360
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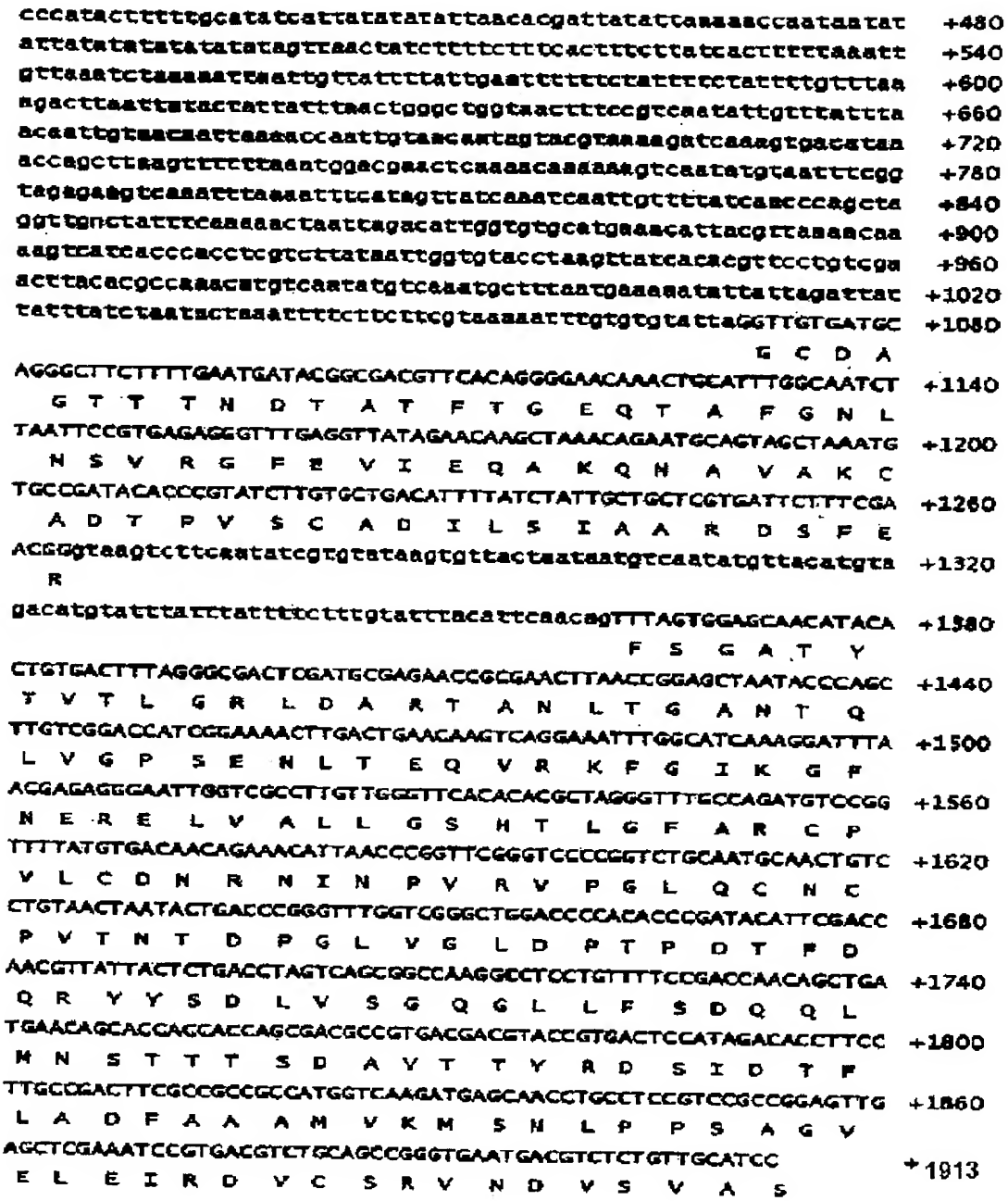
FIG. 2b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N S V R G F E V I E Q A K Q N A V A K C
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A D T P V S C A D I L S I A A R D S F E
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R
gacatgtatttatttatttttctttgtatttacattcaacagTTTAGTGGAGCAACATACA +1380
F S G A T Y
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T V T L G R L D A R T A N L T G A N T Q
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L V G P S E N L T E Q V R K F G I K G F
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V L C D N R N I N P V R V P G L Q C N C
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Q R Y Y S D L V S G Q G L L F S D Q Q L
TGAACAGCACCAGCACCAGCGACGCCGTGACGACGTACCGTGACTCCATAGACACCTTCC +1800
M N S T T T S D A V T T Y R D S I D T F
TTGCCGACTTCGCCGCCGCCATGGTCAAGATGAGCAACCTGCCTCCGTCCGCCGGAGTTG +1860
L A D F A A A M V K M S N L P P S A G V
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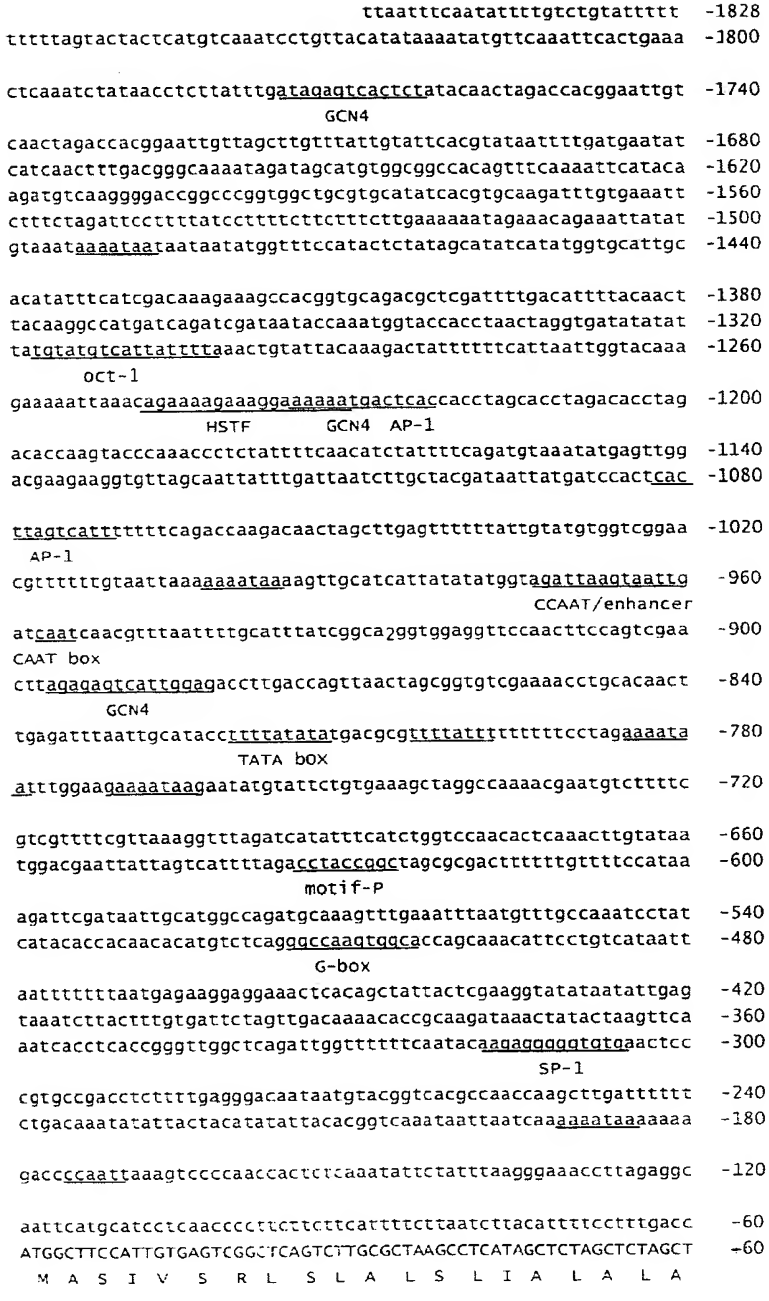
FIG. 3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CAAT box
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G-box
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FIG. 4

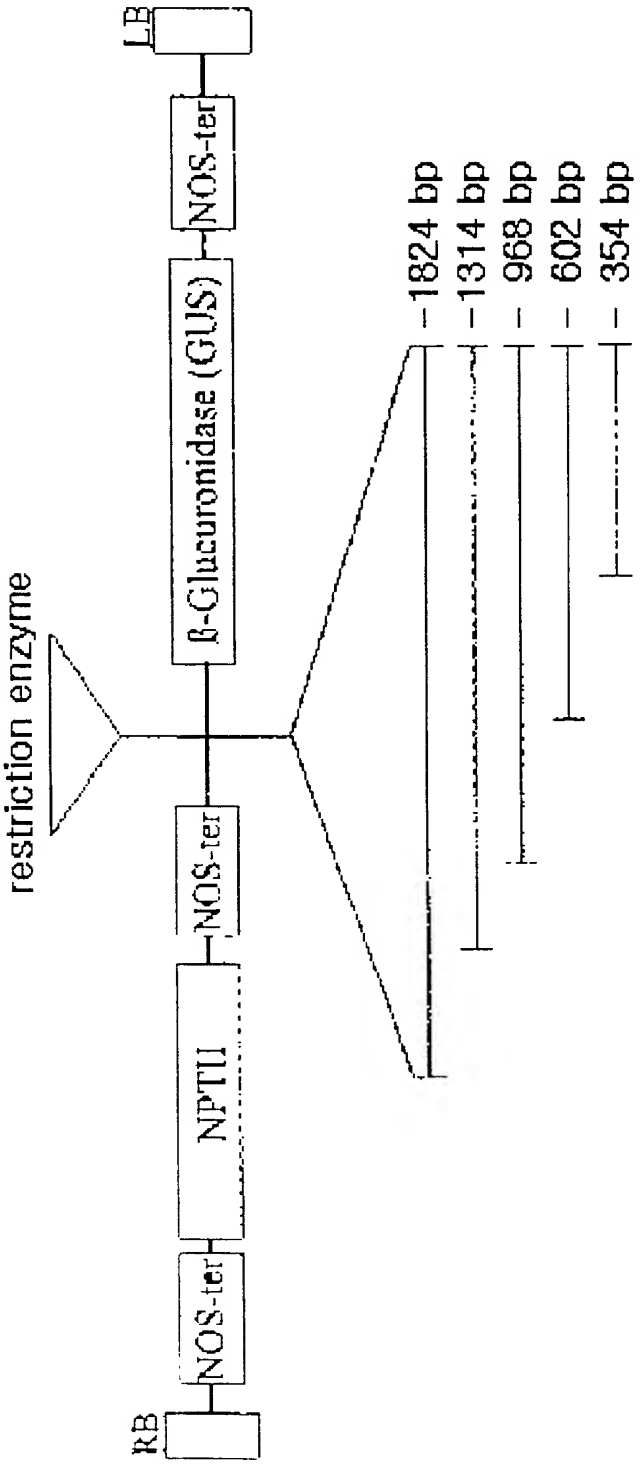

LB
NOS-ter
ß-Glucuronidase (GUS)
restriction enzyme
NOS-ter
NPTII
NOS-ter
RB
1824 bp
1314 bp
968 bp
602 bp
354 bp

FIG. 5

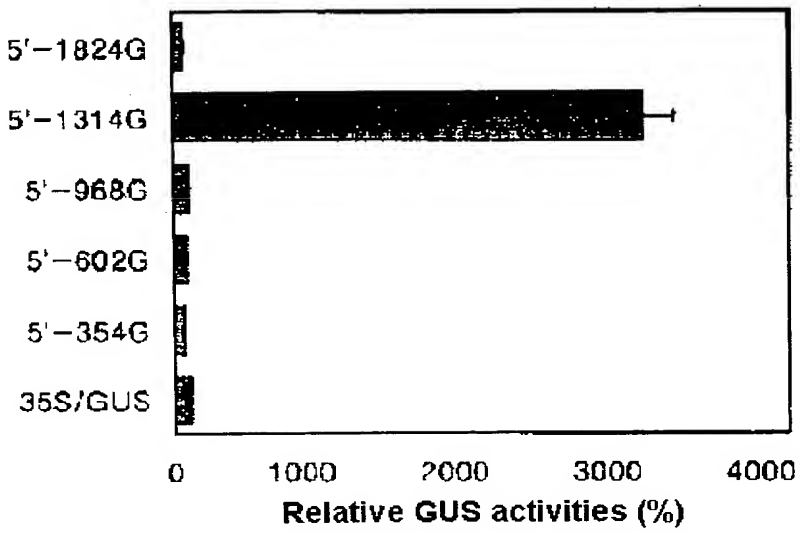
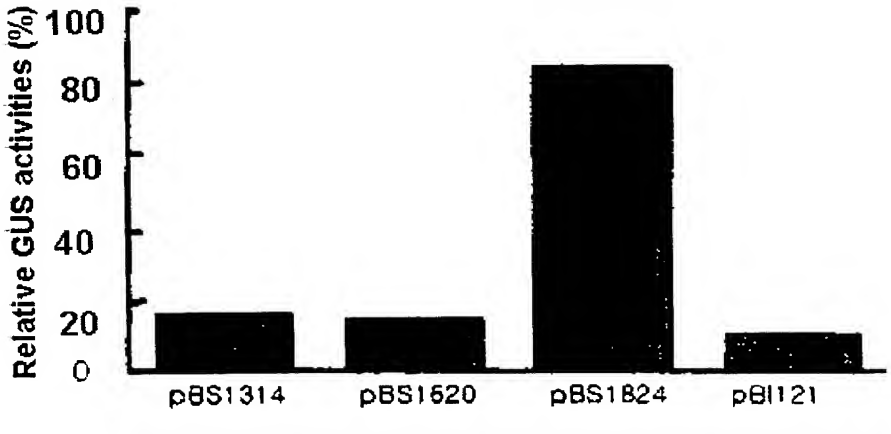

5'-1824G
5'-1314G
5'-968G
5'-602G
5'-354G
35S/GUS
0
1000
2000
3000
4000
Relative GUS activities (%)

FIG. 6


Relative GUS activities (%)
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80
60
40
20
0
pBS1314
pBS1620
pBS1824
pBI121

FIG. 7

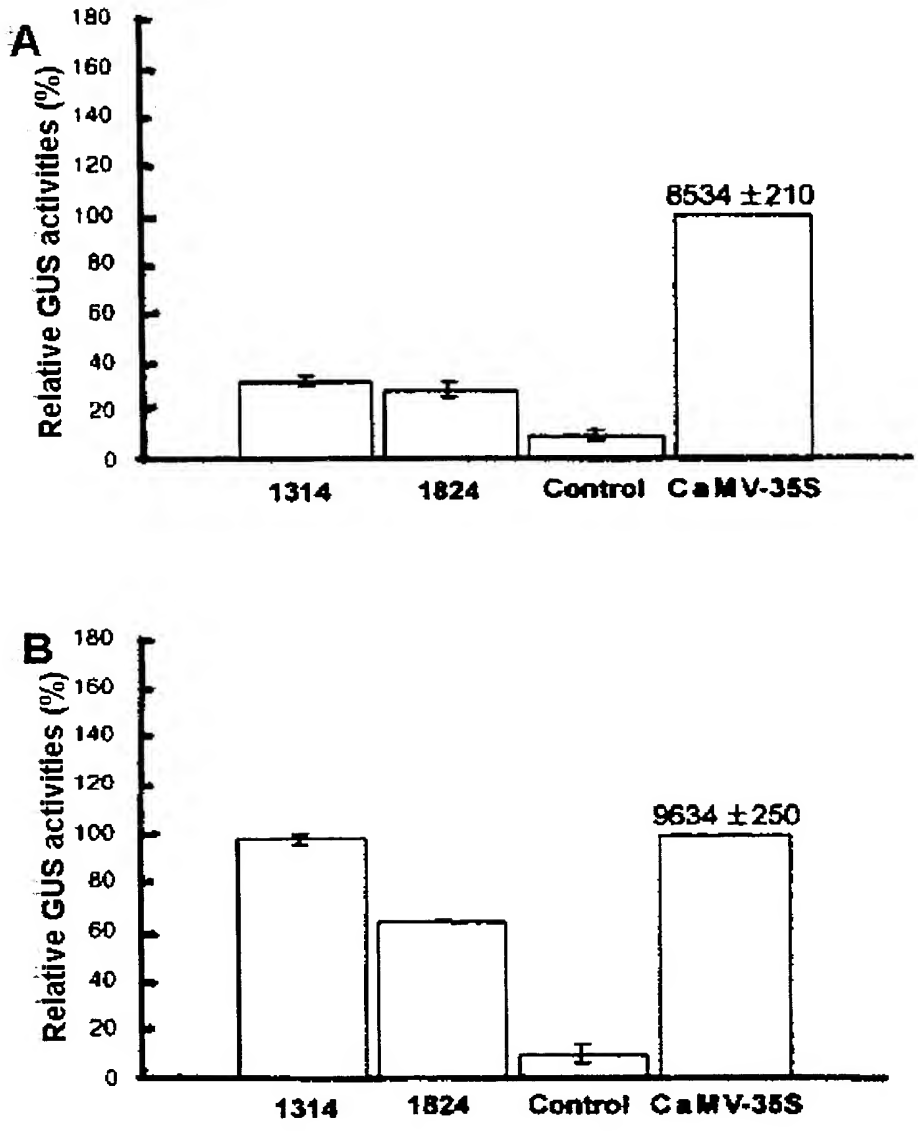
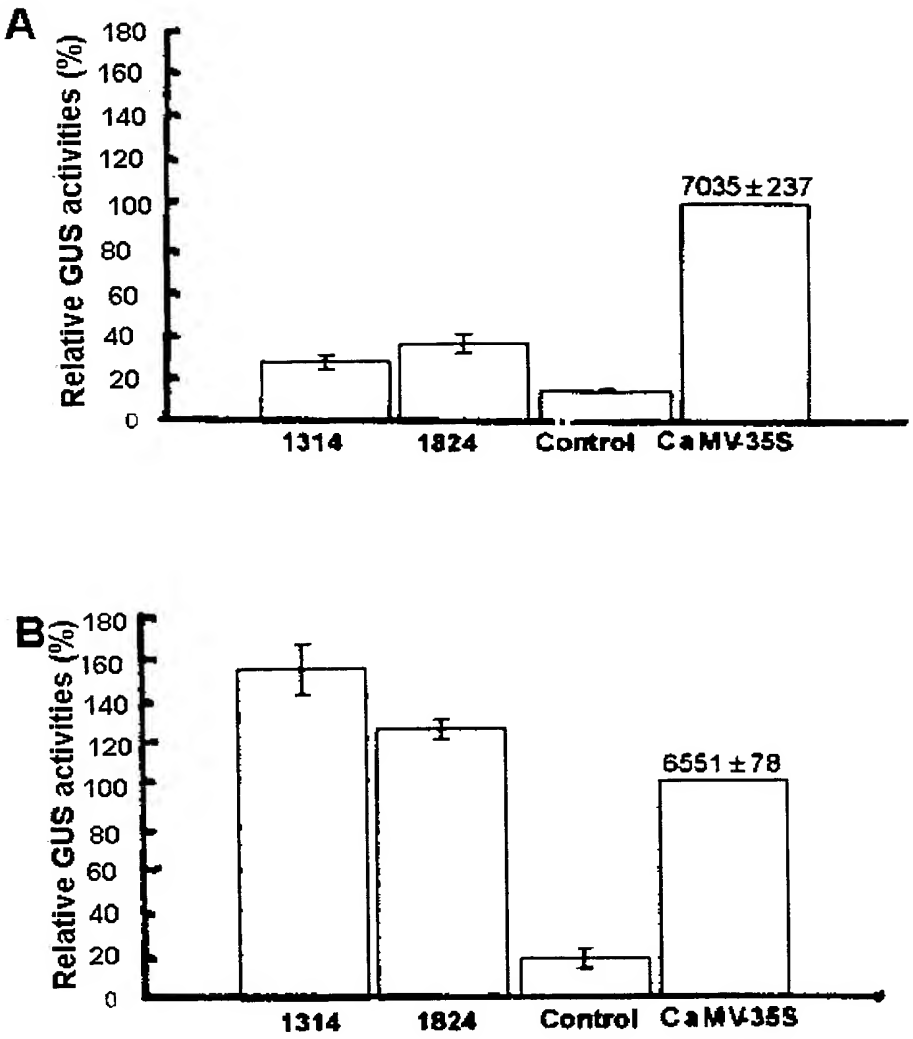

A
Relative GUS activities (%)
180
160
140
120
100
80
60
40
20
0
8534 ±210
1314
1824
Control
CaMV-35S
B
Relative GUS activities (%)
180
160
140
120
100
80
60
40
20
0
9634 ±250
1314
1824
Control
CaMV-35S

FIG. 8


A
Relative GUS activities (%)
180
160
140
120
100
80
60
40
20
0
7035±237
1314
1824
Control
CaMV-35S
B
Relative GUS activities (%)
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100
80
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40
20
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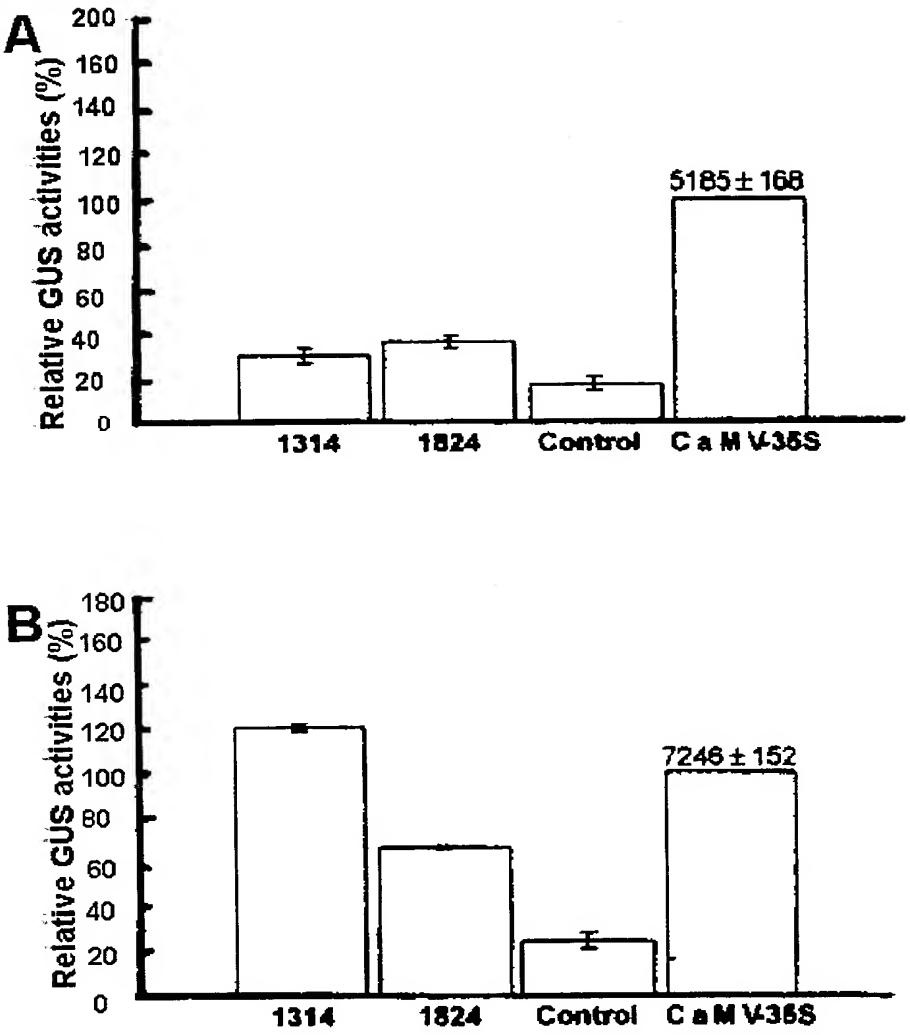
6551±78
1314
1824
Control
CaMV-35S

FIG. 9


A
Relative GUS activities (%)
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160
140
120
100
80
60
40
20
0
5185 ± 168
1314
1824
Control
C a M V-35S
B
Relative GUS activities (%)
180
160
140
120
100
80
60
40
20
0
7246 ± 152
1314
1824
Control
C a M V-35S

FIG. 10a

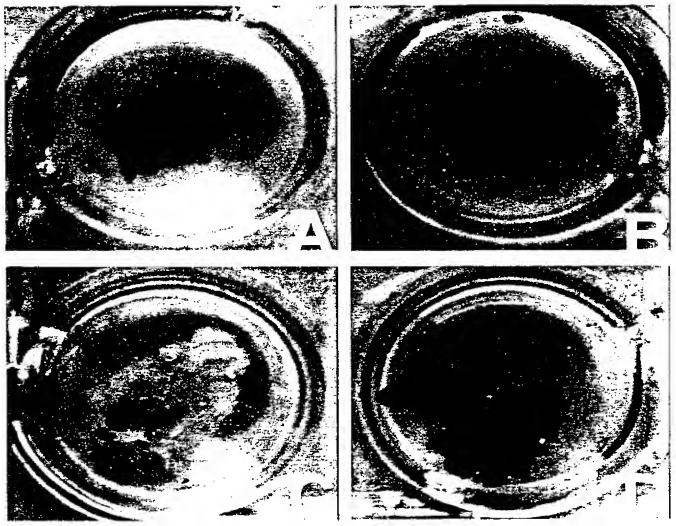


FIG. 10b

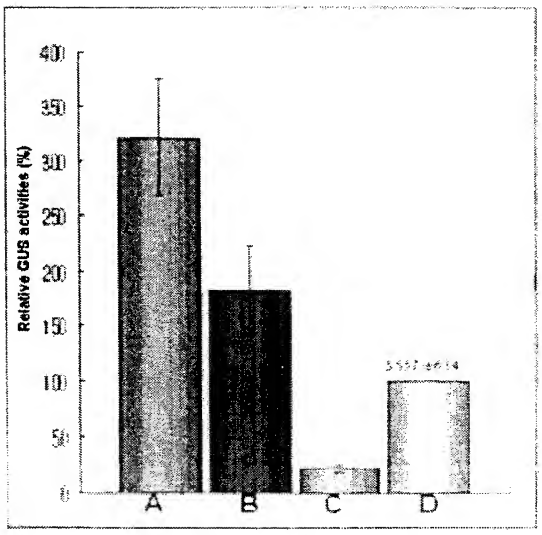


FIG. 11a

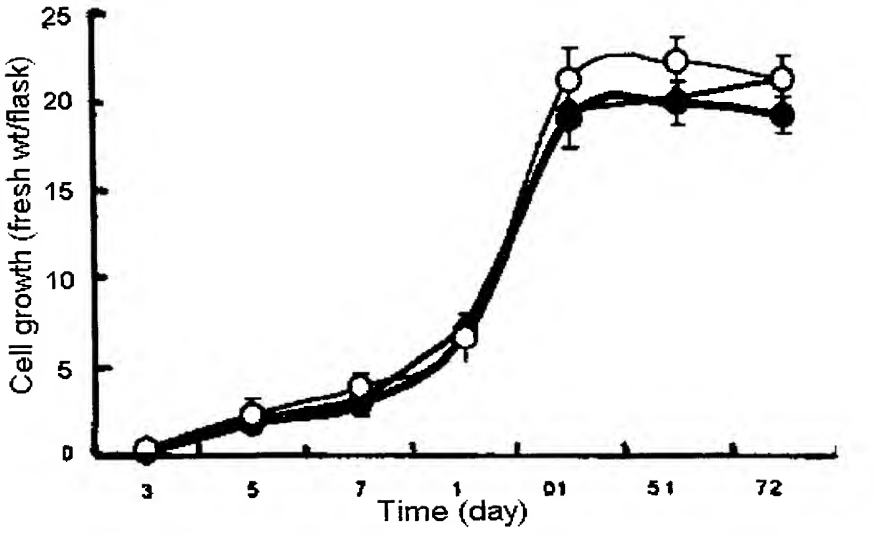
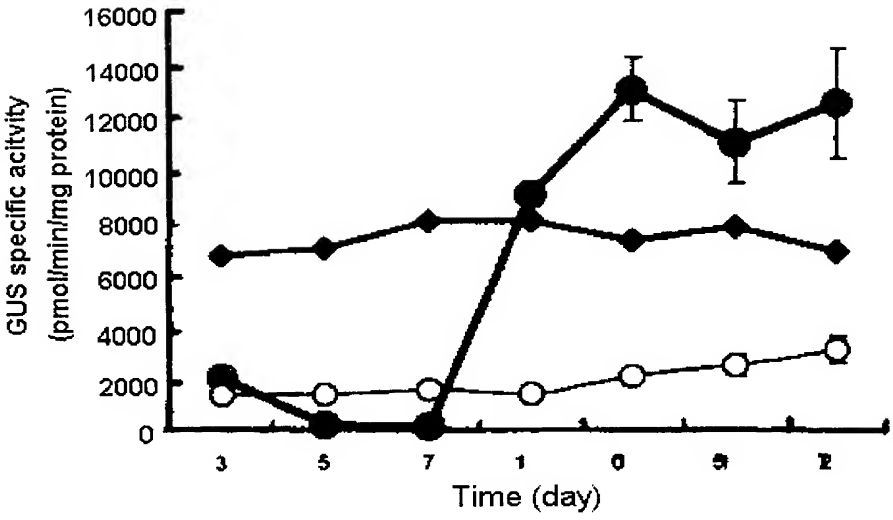


FIG. 11b



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actctataca actagaccac ggaattgtca actagaccac ggaattgtta gcttgtttat 180

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR00/01231

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C12N 15/29

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimun documentation searched (classification system followed by classification symbols)
IPC(7) C12N

Documentation searched other than minimun documentation to the extent that such documents are included in the fileds searched
Korean Patent and application for invensions since 1975

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search trerms used)
IPN, NPS, PAJ, Medline

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,Y	KIM KY et al., "Differential expression of four sweet potato peroxidase genes in response to abscisic acid and ethephon", Phytochemistry, May 2000, 54(1), pages 19-22. see the whole document	1-11
A	HUH GH et al., "Molecular cloning and characterization of cDNAs for anionic and neutral peroxidases from suspension-cultured-cells of sweet potato and their differential expression in response to stress", Mol Gen Genet., July 1997, 255(4), pages 382-391. see the whole document	1-6
A	KIM KY et al., "Molecular characterization of cDNAs for two anionic peroxidases from suspension cultures of sweet potato", Mol Gen Genet., July 1999, 261(6), pages 941-947. see the whole document	1-11
A	WANAPU C et al., cis-regulatory elements of the peroxidase gene in Arabidopsis thaliana involved in root-specific expression and responsiveness to high-salt stress", Ann N Y Acad Sci., May 1996, 782, pages 107-114. see the whole document	1-11

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevence
"E" earlier application or patent but published on or after the international filing date
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 MARCH 2001 (13.03.2001)	13 MARCH 2001 (13.03.2001)

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